

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1 to 55 (canceled)

Claim 56 (currently amended): A ~~eDNA-RNA~~ cDNA-mRNA hybrid comprising a ~~first strand~~ cDNA ~~synthesis~~ hybridised to a RNA wherein the cDNA comprises from the 5' end, an amplifier sequence, 3' to which is an RNA polymerase promoter operably linked to an RNA annealing region, and wherein at least one non-templated nucleotide at the 3' end of the ~~first strand~~ cDNA is hybridised to a template switching oligonucleotide, and wherein ~~the amplifier sequence and~~ the template switching oligonucleotide ~~contain~~ contains the same sequence as the amplifier sequence to allow amplification using a single amplification primer the same sequence.

Claim 57 (currently amended): A ~~eDNA-RNA~~ cDNA-mRNA hybrid according to claim 56, wherein the RNA polymerase promoter is a bacteriophage promoter selected from the group consisting of T7, T3, and SP6.

Claim 58 (currently amended): A ~~eDNA-RNA~~ cDNA-mRNA hybrid according to claim 56, wherein the RNA annealing region comprises poly (dT) of about 10 to about 30 T residues in length.

Claim 59 (currently amended): A ~~eDNA-RNA~~ cDNA-mRNA hybrid according to claim 56, wherein the 3' end of the RNA annealing region comprises a VN clamp, wherein V is A, G or C and N is A, G, C or T.

Claim 60 (currently amended): A cDNA-mRNA hybrid according to claim 56, wherein at least one non-templated nucleotide at the 3' end of the ~~first strand~~ cDNA ~~synthesis~~ is

deoxycytidine.

Claim 61 (currently amended): A cDNA-mRNA hybrid according to claim 56, wherein at least three non-templated nucleotide at the 3' end of the ~~first strand~~ cDNA ~~synthesis~~ are hybridised to a template switching oligonucleotide.

Claim 62 (currently amended): A cDNA-mRNA hybrid according to claim 56, wherein at least three of the non-templated nucleotides at the 3' end of the ~~first strand~~ cDNA ~~synthesis~~ are deoxycytidine nucleotides.

Claim 63 (previously presented): A cDNA-mRNA hybrid according to claim 56, wherein the template switching oligonucleotide has at least three guanine residues at its 3' end.

Claim 64 (currently amended): A cDNA-mRNA hybrid according to claim 56, further comprising a sequence for hybridising an amplification primer, and wherein the amplifier sequence, amplification primer, and the template switching oligonucleotide contain the same sequence to allow PCR amplification using a single amplification primer ~~contains the same sequence as the amplifier sequence and the template switching oligonucleotide.~~

Claim 65 (canceled)

Claim 66 (currently amended): A cDNA-mRNA hybrid according to claim 56, wherein the ~~first strand~~ cDNA ~~synthesis~~ is synthesised by a reverse transcriptase, and wherein the reverse transcriptase lacks RNaseH activity but retains wild-type polymerase activity.

Claims 67-87 (canceled)

Claim 88 (withdrawn-new): A method for amplifying RNA in a sample comprising the

steps of:

(a) incubating an amplification primer with the cDNA-mRNA hybrid according to claim 56 under conditions to generate double stranded amplification products corresponding to the cDNA, such that the cDNA amplification products comprise a double stranded RNA polymerase promoter; and

(b) incubating the cDNA amplification products comprising the double stranded RNA polymerase promoter under conditions that permit *in vitro* transcription to generate amplified RNA.

Claim 89 (withdrawn-new): A method according to claim 88, wherein the cDNA-RNA hybrid is incubated with a reverse transcriptase that adds at least one deoxycytidine residue to the 3' end of the cDNA.

Claim 90 (withdrawn-new): A method according to claim 88 or claim 89, wherein at least three non-templated nucleotides at the 3' end of the cDNA are hybridised to a template switching oligonucleotide.

Claim 91 (withdrawn-new): A method according to claim 90, wherein at least three of the non-templated nucleotides at the 3' end of the cDNA are deoxycytidine residues.

Claim 92 (withdrawn-new): A method according to claim 91, wherein the reverse transcriptase lacks RNaseH activity but retains wild-type polymerase activity.

Claim 93 (withdrawn-new): A method according to claim 91, wherein the template switching oligonucleotide comprises at least three ribonucleotide residues.

Claim 94 (withdrawn-new): A method according to claim 92, wherein the template switching oligonucleotide comprises at least three guanine residues.

Claim 95 (withdrawn-new): A method according to claim 94, wherein said amplification primer has the same sequence as the amplifier sequence for synthesizing the cDNA.

Claim 96 (withdrawn-new): A method according to claim 95, wherein the double stranded amplification products are obtained by PCR.

Claim 97 (withdrawn-new): A method according to claim 96, wherein the optimum number of cycles to generate the double stranded amplification products is determined by a method comprising the steps of:

- (a) providing a plurality of samples with a known amount of RNA;
- (b) performing amplification for a defined number of cycles on the plurality of samples;
- (c) purifying the double stranded amplification products;
- (d) providing for the *in vitro* transcription of the purified amplification products; and
- (e) determining the number of amplification cycles that results in the minimum amount of amplified RNA that is required.

Claim 98 (withdrawn-new): A method according to claim 97, wherein the RNA sample is a clinical sample selected from the group consisting of a biopsy, a microdissected tissue, a fine needle aspirate, a flow-sorted cell, a laser captured microdissected cell or a single cell.

Claim 99 (withdrawn-new): A method for preparing an expression library of a cell or a cell population comprising the steps of:

- (a) incubating an amplification primer with the cDNA-mRNA hybrid according to claim 56 under conditions that generate double stranded amplification products corresponding to the cDNA, such that the double stranded cDNA amplification products comprise a double stranded RNA polymerase promoter; and

(b) incubating said double stranded cDNA amplification products comprising said double stranded RNA polymerase promoter under conditions that permit *in vitro* transcription to generate amplified RNA.

Claim 100 (withdrawn-new): A method of preparing a cDNA library from a collection of mRNA molecules comprising:

(a) incubating a PCR primer with the cDNA-mRNA hybrid according to claim 56 under conditions that generate double stranded amplification products corresponding to the cDNA, such that the double stranded cDNA amplification products comprise a double stranded RNA polymerase promoter;

(b) incubating said double stranded cDNA amplification products comprising said double stranded RNA polymerase promoter under conditions that permit *in vitro* transcription to generate amplified RNA; and

(c) preparing a cDNA library from the amplified RNA.

Claim 101 (withdrawn-new): A method for performing subtractive hybridisation comprising:

(a) incubating an amplification primer with said cDNA-mRNA hybrid according to claim 56 under conditions to generate double stranded amplification products corresponding to the cDNA, such that the double stranded cDNA amplification products comprise a double stranded RNA polymerase promoter;

(b) incubating said double stranded cDNA amplification products comprising said double stranded RNA polymerase promoter under conditions that permit *in vitro* transcription to generate amplified RNA;

(c) contacting said amplified RNA with a single stranded nucleic acid population in the opposite sense to said amplified RNA;

(d) providing for the hybridisation of the sequences present in the amplified RNA and the single stranded nucleic acid population; and

(e) isolating the nucleic acid population that remains single stranded.

Claim 102 (withdrawn-new): A method for detecting the expression of a gene of interest comprising:

(a) incubating an amplification primer with said cDNA-mRNA hybrid according to claim 56 under conditions to generate double stranded amplification products corresponding to the cDNA, such that the double stranded cDNA amplification products comprise a double stranded RNA polymerase promoter;

(b) incubating said double stranded cDNA amplification products comprising said double stranded RNA polymerase promoter under conditions that permit *in vitro* transcription to generate amplified RNA; and

(c) determining the presence or absence of amplified RNA, which amplified RNA is complementary to mRNA corresponding to the gene of interest.

Claim 103 (withdrawn-new): A kit for the amplification of RNA in a sample comprising:

(a) the cDNA-mRNA hybrid according to claim 56; and

(b) an amplification primer that has the same sequence as the template switching oligonucleotide to allow amplification using a single amplification primer.

Claim 104 (withdrawn-new): The kit according to claim 103, wherein the kit further comprises in a separate container a reverse transcriptase, wherein the reverse transcriptase lacks RNaseH activity but retains wild-type polymerase activity.

Claim 105 (withdrawn-new): The kit according to claim 103 or 104, wherein the kit further comprises in a separate container an RNA polymerase specific to the RNA polymerase promoter of the cDNA.

Claim 106 (withdrawn-new): The kit according to claim 105, wherein the RNA

polymerase promoter is selected from a T7, T3 or SP6 RNA polymerase promoter.

Claim 107 (withdrawn-new): The kit according to claim 106, wherein the kit further comprises an amplification buffer and one or more amplification enzymes, wherein the amplification buffer and the amplification enzyme(s) are PCR amplification buffer and PCR amplification enzyme(s).

Claim 108 (withdrawn-new): The method of claim 88, wherein the amplified RNA is an antisense RNA.